

Remarks

We respectfully request that the above-identified amendments entered into the file of the case. They are made to correct typographical and grammatical errors and to insert sequence ID numbers into the Specification. No new matter has been added.

An early action on the merits of the case is respectfully requested.

Respectfully submitted,



T. Daniel Christenbury
Reg. No. 31,750

TDC:gj
(215) 563-1810

1003 to 224186

In the Specification (Marked up version)

Please replace the paragraph 8 on page 12 with the following:

Figure 11 shows integration of the mer operon into the chloroplast genome. A: PCR using specific primers that land in the gene cassette (5P(SEQ ID NO: 5)/2M (SEQ ID NO: 6)) show a product of 3.8kb size (clones 2, 4, 5, 7, 9, 11). Clones 1 and 3 show no integration of the cassette. Positive control, is plasmid pLD-merAB-3'UTR. Negative control is untransformed plant DNA. B: PCR using specific primers that land within the native chloroplast genome (3P/3M), eliminate mutants (clone 3), showing integration of the cassette into the chloroplast genome (clones: 1, 2, 4, 5, 6, 7, 9, 11. 1.6 kb PCR product).

Please replace the paragraph bridging pages 19 and 20 with the following:

Chaperonins are a class of a protein referred to as chaperones which has been shown to consist of helper proteins in chain folding and assembly with the cells (Gierasch and King, 1990). They facilitate the folding and assembly of newly synthesized polypeptide chains into functional three-dimensional structures by preventing off-pathway reactions during folding that lead to aggregation (Agashe VR et. al. 2000). Chaperonins provide a sequestered environment in which folding can proceed unimpaired by intermolecular interactions between non-native polypeptides (Agashe VR et. al. 2000). Those skilled in the art will be familiar with the E. Coli chaperonins: groEL and groES (Viitanen PV et. al. 1995), (Gierasch and King, 1990). Plant chaperonins chaperonin-60 and chaperonin-10, which are homologous of gro-EL and gro-ES, respectively. ~~Homologous~~Homologues of the E. Coli groEL and groES continue to be identified. For instance, a stable complex of the chaperonins has been isolated and crystallized from the extremely thermophilic bacterium *Thermus thermophilus* (Lissin NM et. al. 1992). Likewise, plant chaperonins – located both

in plastids and the cytosol, continue to be identified (Baneyx F. et. al., 1995; Viitanen PV et. al., 1995; Burt WJ et. al. 1994, Grellet F. et. al. 1993; Bertsch U et. al., 1992). These articles are hereby incorporated in their entirety by reference.

Please replace second paragraph on page 25 with the following:

This invention enables expression of polycistrons in the chloroplast genome. In contrast to prior efforts in engineering gene expression in transgenic plants, the present invention allows for this achievement in a single transformation event that is environmentally safe. The invention thus opens the possibility for gene pyramiding: the insertion of multiple insecticidal genes. The invention contemplates operons which include not only *Bacillus thuringiensis* (B.t.) insecticidal toxin genes, but also non-B.t. insecticidal toxin genes such as cholesterol oxidase, alpha-amylase inhibitors, protease inhibitors, the cowpea trypsin inhibitors, and the potato proteinase inhibitor II. Inclusion of multiple heterologous insecticidal toxin genes retards the ability of insects to develop resistance to bio-pesticides.

Please replace the third paragraph on page 25 with the following:

Further, this invention provides a method of engineering biological pathways into the chloroplast genome in a single transformation event that is environmentally safe. Because gene expression is controlled by one promoter, DNA sequences encoding the different genes necessary in a pathway can be co-expressed to the same levels. Once expressed, the genes of the pathway can act in concert. Gene expression can result in synthesis of enzymes that confer desired traits such as degradation of metal ions, herbicides, pesticides, solvents, ~~toluene~~toluene, naphthalene, and other xenobiotics. An example is the chloroplast

transformation of plant chloroplasts with the Mer operon leading to the biodegradation of mercury and organomercurials. Other pathways include the pigment biosynthesis pathway, biosynthetic pathways for enzymes that are could confer desired traits such as degradation of xenobiotic compounds noted above, pathways for amino acids such as the lysine biosynthetic pathway, and pathways for the synthesis of vitamins, carbohydrates, fatty acids, biopolymers and polyesters. Further examples are provided in chapters 12 and 13 of Molecular Biotechnology by Glick and Pasternak, which is herein incorporated by reference. Other xenobiotics which can be degraded using the system of this invention include those given in U.S. patent 4,259,444 to Chakrabarty which is herein incorporated by reference.

Please replace paragraph 4 on page 27 with the following:

PCR Analysis: DNA was extracted from leaves using the QIAGEN DNeasy Plant Mini Kit. PCR was done using the Perkin Elmer Gene Amp PCR System 2400. All PCR reactions were performed using the Qiagen Taq DNA Polymerase Kit. Primer sequences used were: 1P (SEQ ID NO: 1) (5'-ACAATGTAGCCGTACTGGA AGGTGCG GGTG-3'), 1M (SEQ ID NO: 2) (5'-CGCGCTT AGC TGGATAACGCCACGGAA-3'), 3P(SEQ ID NO: 3)(5'-AAAA CCCGTCCTCA GTTCGGATTGC-3'), and 3M (SEQ ID NO: 4) (5'-CCGCGTTGTTTCATCAA GCCTTACG-3'). Samples were run for 30 cycles with the following sequence: 94°C for 1 minute, 70°C for 1.5 minutes, and 72°C for 3 minutes. PCR products were separated on 0.8% agarose gels.

Please replace the last paragraph on page 35 with the following:

Begley TPA, Walts AE, Walsh CT (1986) Mechanistic studies of a protonolytic

~~organomercurial~~organomercurial cleaving enzyme: bacterial organomercurial lyase.

Biochemistry 25: 7192-7200.

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Marked Up Version in the Claims

1. (Amended) A stable chloroplast transformation and expression vector which is capable of introducing multiple genes into a selected plant by a single integration event, wherein each step of said multiple genes is carried out by an enzyme encoding a heterologous DNA sequence which comprises an expression cassette, comprising as operably linked components, in the 5' to the 3' direction of translation, a promoter operative in said plastids which drives a multi-gene operon, a selectable marker sequence, the multi-gene operon which is functional to co-express multiple enzymes in the plastids, a transcription termination region functional in said plastids, and flanking each side of the expression cassette, flanking DNA sequences which are ~~homomologous~~homologous to DNA sequences inclusive of a spacer sequence of the target plastid genome, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated throughout homologous recombination of the flanking sequence with the homologous sequences in the target plastid gene.

28. (Amended) A method of transforming a chloroplast of a selected plant species or the progeny thereof to confer greater resistance to metal ions than the corresponding parental plant which does not require several back crosses to create complete pathway that detoxifies mercury and ~~organomercurial~~organomercurial, said method comprising the steps of:

stably transforming the chloroplast of a plant by inserting an expression cassette containing the mercury resistance coding sequences of claim 21 into a plant species or the progeny thereof, growing the transforming plant species under conditions which allow

